12-10-2020

Altered Toxin Binding or Access to the Binding Site, What Changes in the Tetrodotoxin Resistant Sodium Channels of Garter Snakes?

Kenyon Mitchell
Utah State University, kenyon.mitchell@aggiemail.usu.edu

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Kenyon Mitchell
Utah State University

Shana Geffeney, PhD
Utah State University

Introduction

Voltage-gated sodium channels are transmembrane proteins that are required for the generation and propagation of action potentials in excitable vertebrate tissue such as muscles and nerves. TTX binds to and blocks sodium channel function.

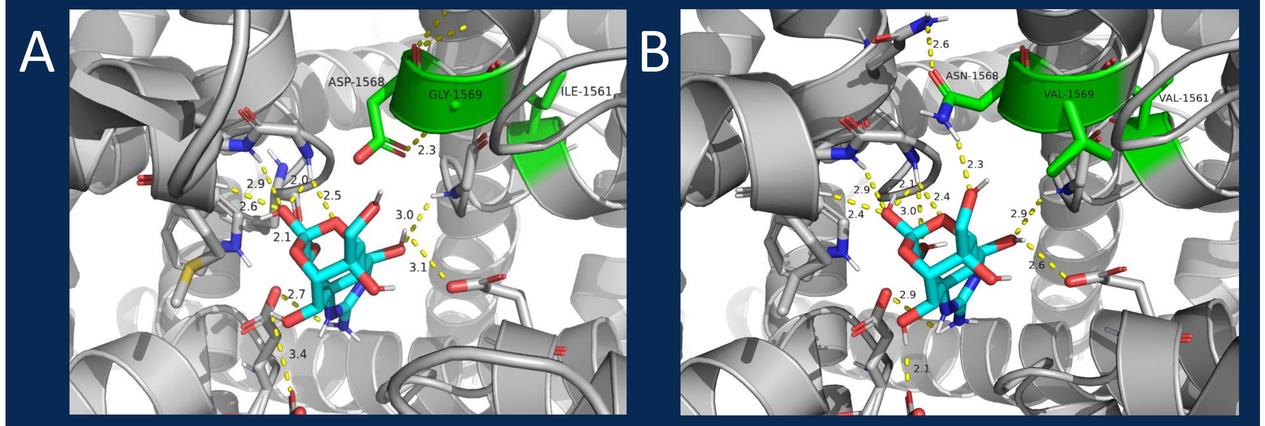
- **Target Residues:** Four substitution mutations have been identified in *T. sirtalis* $\mathrm{Na_V}1.4$ channels that result in TTX resistance: G1569V, D1568N, I1561V, I1556L. All four substitution are in the TTX binding site.
- **Magnitude of Resistance:** With all four substitutions, *T. sirtalis* $\mathrm{Na_V}1.4$ channel are 100x more resistant to TTX binding than TTX-sensitive snake channels.
- **Hypothesis:** Resistance is achieved by reducing binding affinity for TTX of the pore binding site, specifically by the substitution D1568N in TTX-resistant snakes.

Does altered TTX binding affinity explain why we see almost two orders of magnitude of greater resistance in TTX-resistant snake channels?

Methods

1. Homology models of *T. sirtalis* $\mathrm{Na_V}1.4$ were constructed using Swiss-Model, the model with the best QMEAN scores was selected for analysis.
2. PyMol 2.0 was used to alter amino acids at four target residue locations in the homology model.
3. AutoDock and AutoDock Vina were used to model ligand binding. Autodock Vina was used to calculate mean binding affinity.

Figure 1 – *T. sirtalis* $\mathrm{Na_V}1.4$ with TTX docked in pore.

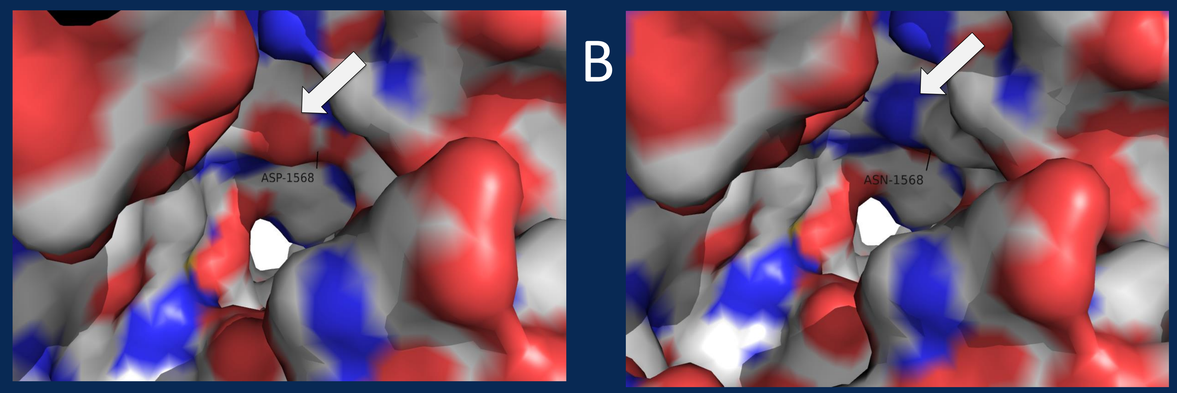


Extracellular view of pore. Figure 1.A shows TTX docked in the wild type, TTX sensitive channel. Figure 1.B shows TTX docked in the mutant TTX resistant channel. The four target residues are shown in green. Hydrogen bonds are shown by yellow dashed lines, associated measurements are given in angstroms.

Table 1- Binding Affinity

	Mean Binding Affinity (kcals/mol)	Standard Deviation
TTX Sensitive	-6.17778	0.44378
TTX Resistant (M1-M4)	-6.56667	0.33912

Figure 2 – Surface View of the Pore



Extracellular view of pore. Red areas represent oxygen and their associated partial negative charges. Blue areas represent nitrogen with their associated partial positive charges. Figure 2.A shows the surface of the inner pore on the wild type, TTX sensitive channel. Figure 1.B shows the surface of the mutant TTX resistant channel.

Results

- Measured mean binding affinity for TTX increased for TTX-resistant snake channels compared to TTX-sensitive snake channels (Table1), these results do not support our hypothesis.
- Nine hydrogen bonds were identified between TTX and the TTX-sensitive channel (Fig. 1A) and ten hydrogen bonds were identified between TTX and the TTX-resistant channel (Fig. 1B) This may explain why we see higher binding affinity for TTX in the resistant channel (Table1).
- A partial negative charge on D1568 (TTX-sensitive) and a partial positive charge on N1568 (TTX-resistant) point directly into the pore (Fig. 2A & 2B).

Conclusions

1. When TTX is positioned at the TTX binding site, amino acid substitutions from TTX-resistant snakes do not reduce TTX binding affinity.
2. Instead, the change in charge from a partial negative on D1568 (TTX-sensitive) to a partial positive on N1568 (TTX-resistant) may provide a barrier for positively-charged TTX moving deeper in the pore and entering the binding site (Fig.2.A and Fig.2.B).

Future Directions

1. We will improve our model (e.g. energy minimization) using DeepView, Swiss-PDB Viewer.
2. We will use Poisson-Boltzmann calculations to measure the probability of TTX entering the binding site in TTX-sensitive and TTX-resistant snake channels.
3. We will measure TTX on- and off-rates using electrophysiological recordings of TTX-sensitive and TTX-resistant snake channels.

Utah State University

Study conducted with funding from Uintah Basin Undergraduate Research Internship Program.
We would like to thank Dr. James Taylor for continuing to support this program.

Kenyon Mitchell
Utah State University
Department of Biology
Kenyon.Mitchell@aggiemail.usu.edu